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Acute tolerance of juvenile Florida pompano, *Trachinotus carolinus* L., to ammonia and nitrite at various salinities

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Abstract

The acute tolerance of juvenile Florida pompano *Trachinotus carolinus* L. (mean weight ± SE = 8.1 ± 0.5 g) to environmental unionized ammonia-nitrogen (NH_3-N) and nitrite-nitrogen (NO_2-N) at various salinities was determined via a series of static exposure trials. Median-lethal concentrations (LC_{50} values) of NH_3-N and NO_2-N at 24, 48, and 96 h of exposure were calculated at salinities of 6.3, 12.5 and 25.0 g L^{-1} at 28 °C (pH = 8.23–8.36). Tolerance of pompano to acute NH_3-N exposure was not affected by salinity, with 24, 48 and 96 h LC_{50} values ranging from 1.05 to 1.12, 1.00 to 1.08 and 0.95 to 1.01 mg NH_3-N L^{-1} respectively. Regarding NO_2-N, tolerance of pompano to this environmental toxicant was compromised at reduced salinities. Median-lethal concentrations of NO_2-N to pompano at 24, 48 and 96 h of exposure ranged from 67.4 to 220.1, 56.9 to 140.7 and 16.7 to 34.2 mg NO_2-N L^{-1} respectively. The results of this study indicate that juvenile Florida pompano are relatively sensitive to acute NH_3-N and NO_2-N exposure, and in the case of the latter, especially at lower salinities.

Keywords: Florida pompano *Trachinotus carolinus*, L., ammonia, nitrite, salinity

Introduction

Owing to their excellent flavour and limited supply, the market value of Florida pompano, *Trachinotus carolinus* L. (Carangidae), exceeds that of many marine finfish, with ex-vessel prices of whole fish ranging from US$7 to $13 kg^{-1} (NMFS 2004). As such, efforts to develop culture methods for this species have intensified in recent years. While research has been conducted on topics including reproduction, larviculture, nutrition and ongrowing procedures (Watanabe 1995; Craig 2000), relatively little information exists with respect to the environmental requirements of pompano and their tolerance to culture conditions.

Because of land cost, effluent discharge regulations and restrictions on use of coastal and offshore waters, it is likely that future expansion of mariculture in the United States will occur using land-based recirculating or limited flow-through production formats (Weirich, Segars, Bruce & Browdy 2003). However, one problem inherent to such culture systems is the potential development of elevated concentrations of environmental unionized ammonia-nitrogen (NH_3-N) and nitrite-nitrogen (NO_2-N). Although toxic levels of NH_3-N and NO_2-N to many freshwater finfish have been determined (Russo & Thurston 1977; Russo 1984; Lewis & Morris 1986; Tomasso 1994; Stickney 2000; Randall & Tsui 2002; Tomasso & Grosell 2005), considerably less information exists for marine species (Tucker 1998). Regarding pompano, no previous studies have been conducted to determine the tolerance of this species to environmental NH_3-N or NO_2-N. Preliminary observations made at our research facility, coupled with anecdotal reports, suggest that pompano tolerate a wide range of environmental salinities. Because of this and the fact that salinity has been demonstrated to modulate NH_3-N and NO_2-N toxicity in several marine species (Sousa, Meade & Wolke 1974; Crawford & Allen 1977; Alabaster, Shurben & Knowles 1979; Saroglia, Scarano & Tibaldi 1981; Harader & Allen 1983; Wise

& Tomasso 1989; Weirich, Tomasso & Smith 1993; Bianchini, Wasielesky & Miranda-Filho 1996; Sampaio, Wasielesky & Miranda-Filho 2002), the present study was conducted to determine the acute tolerance of juvenile Florida pompano to NH_3-N and NO_2-N exposure at various salinities. Specifically, median-lethal concentrations (LC_{50} values) of NH_3-N and NO_2-N at 24, 48 and 96 h of exposure were determined at salinities of 6.3, 12.5 and 25.0 g L^{-1}.

Materials and methods

Water sources

Water used in exposure trials was obtained from two sources located on the campus of Harbor Branch Oceanographic Institution, Ft Pierce, FL, USA. Saline water was pumped from a 7 m deep well adjacent to the Indian River Lagoon and brackish water was obtained from a 600 m deep well. Both water sources were subjected to biological and mechanical filtration before entry into experimental units. The salinity of the saline and brackish water sources ranged from 30.5 to 32.2 g L^{-1} and from 0.8 to 1.1 g L^{-1} respectively.

Experimental animals and acclimation procedures

Juvenile pompano were purchased from Maritech, Sebastian, FL, USA.[1] Fish were held in a recirculating system consisting of two, 1600 L circular tanks for at least 1 month before initiation of experiments. Fish were maintained at 26–29 °C. Salinity was maintained at 28–30 g L^{-1} by addition of saline and brackish water. During the holding period, fish were fed a 46% protein, 18% lipid 1.5 mm sinking pelleted diet (Melick Aquafeeds, Catawissa, PA, USA) at a rate of 3% body weight day^{-1} divided into two feedings (09:00 and 16:00 hours). To ensure elimination of naturally occurring gill parasites and monogenetic trematodes, fish were treated with copper sulphate (0.25 mg L^{-1} as Cu^{2+}; Cutrine®-Plus Algaecide/Herbicide, Applied Biochemists, Milwaukee, WI, USA) and praziquantel (2.5 mg L^{-1}; PondRx.com, Morganton, GA, USA), respectively, throughout the holding period. Before the initiation of each exposure trial, sufficient fish were removed from holding tanks and placed in a single 1600 L tank containing saline water of the same salinity as that of the holding tanks. Over the course of 72 h fish were acclimated to target salinities via the gradual addition of brackish water. Feed was withheld during the acclimation period. The mean weight ± SE ($N = 100$) of pompano subjected to experiments was 8.1 ± 0.5 g.

[1]The use of trade, firm or corporation names in this publication is for the information and convenience of the reader. Such use does not constitute an official endorsement or approval by the United States Department of Agriculture or the Agricultural Research Service of any product or service to the exclusion of others that may be suitable.

Experimental systems, design and protocol

Experiments were conducted using 100 L cylindrical polyethylene tanks (Polytank, Litchfield, MN, USA) operated under static conditions. Tanks were housed in a laboratory maintained under controlled temperature and continuous light conditions. The light level at the water surface of tanks ranged from 300 to 400 lx (EasyView™ light meter, Extech Instruments, Waltham, MA, USA). Blown air was supplied to each tank and temperature was maintained at 28 °C with Ebo-Jaeger® 75-watt immersion heaters (Aquatic Ecosystems, Apopka, FL, USA). A total of six exposure trials were conducted to evaluate acute tolerance of fish to NH_3-N and NO_2-N at three nominal salinity levels (6.3, 12.5 and 25.0 g L^{-1}). Each exposure contained a control (nominal NH_3-N or NO_2-N = 0 mg L^{-1}) and five exposure concentrations of either NH_3-N or NO_2-N, achieved through the addition of ammonium chloride or sodium nitrite (Sigma Chemical, St. Louis, MO, USA) respectively. Exposure concentrations increased from low to high levels via geometric progression. Nominal NH_3-N and NO_2-N exposure concentrations ranged from 0.70 to 2.05 mg L^{-1} and from 9 to 231 mg L^{-1} respectively. Four replicates existed for the control and each exposure concentration. Treatments (exposure concentrations) were assigned to experimental tanks using a completely randomized design. Before initiation of each trial, tanks were sterilized with chlorine bleach and rinsed. Subsequently, 60 L of water was added to each tank from a common reservoir filled with water of the appropriate salinity. Ammonium chloride or sodium nitrite was added to achieve exposure concentrations, and five fish were stocked 24 h later in each tank. Fish were observed twice daily at 08:00 and 20:00 hours, and mortalities were counted and removed. Feed was withheld throughout each exposure trial. Median-lethal concentrations (LC_{50} values) at 24, 48 and 96 h were calculated from each

replicate series of tanks using the trimmed Spearman–Karber method (Hamilton, Russo & Thurston 1977).

Water quality

Temperature, dissolved oxygen, salinity, pH, total ammonia-nitrogen (TA-N) and NO_2-N of each experimental tank were measured daily at 09:00 and 17:00 hours during each exposure trial. Three samples of water were obtained at the initiation of each exposure trial for determination of calcium, chloride and alkalinity levels at each salinity tested. These variables were also determined for both water sources used to formulate experimental salinities. Temperature and dissolved oxygen were measured with a YSI Model 85 meter (Yellow Springs Instrument, Yellow Springs, OH, USA). Total TA-N, NO_2-N and Cl^- were determined via colorimetric assays (methods 8155, 8153 and 8113 respectively) using a D/R 2500 spectrophotometer (Hach, Loveland, CO, USA). The pH was measured with a Hach sensION^{TM} pH probe attached to the spectrophotometer. Calcium and alkalinity were determined using Hach digital titration methods 8204 and 8203 respectively. NH_3-N levels were determined from TA-N, temperature, salinity and pH values using the formulae and tables presented in Emerson, Russo, Lund and Thurston (1975), Bower and Bidwell (1978) and Soderberg and Meade (1991). Water quality data measured daily during NH_3-N and NO_2-N exposure trials are presented in Table 1. Temperature, salinity and NH_3-N and NO_2-N exposure concentrations were maintained within 10% of nominal values. During NH_3-N exposure trials, NO_2-N concentrations of control and exposure tanks remained below 0.3 mg L^{-1}. During NO_2-N exposure trials, NH_3-N concentrations of control and exposure tanks remained below 0.2 mg L^{-1}. Calcium, chloride and alkalinity levels of saline and brackish water sources and nominal salinities tested in exposure trials are presented in Table 2. Chloride levels of all nominal salinities and calcium levels of nominal salinities > 20 g L^{-1} approximated those expected from diluted natural seawater (Spotte 1979). Calcium concentrations of nominal salinities < 20 g L^{-1} were slightly elevated due to the use of brackish water as a dilutant.

Statistical analyses

Statistical analyses of calculated LC_{50} values were performed using SIGMASTAT® Version 3.0 software (SPSS, Chicago, IL, USA). All data were subjected to

Table 1 Values (means ± SE) for water quality parameters measured during 96 h exposure of juvenile Florida pompano, *Trachinotus carolinus* L., to ammonia-nitrogen (NH_3-N) and nitrite-nitrogen (NO_2-N) at various salinities

Toxicant	Nominal Salinity (g L^{-1})	Actual Salinity (g L^{-1})	Temperature (°C)	DO* (mg L^{-1})	pH	NH_3-N or NO_2-N (% nominal)
NH_3-N	25.0	25.0 ± 0.0	27.9 ± 0.0	6.2 ± 0.0	8.36 ± 0.01	94.8 ± 1.3
	12.5	11.6 ± 0.0	27.7 ± 0.0	6.7 ± 0.0	8.36 ± 0.01	100.2 ± 1.0
	6.3	5.8 ± 0.0	27.8 ± 0.0	6.8 ± 0.0	8.33 ± 0.06	102.4 ± 1.3
NO_2-N	25.0	26.4 ± 0.0	27.9 ± 0.0	5.7 ± 0.0	8.23 ± 0.00	100.0 ± 0.8
	12.5	13.3 ± 0.0	28.0 ± 0.0	6.3 ± 0.0	8.33 ± 0.01	97.2 ± 0.7
	6.3	6.7 ± 0.0	28.0 ± 0.0	6.5 ± 0.0	8.35 ± 0.00	101.7 ± 1.0

*Dissolved oxygen.

Table 2 Calcium, chloride and alkalinity levels (means ± SE) of saline and brackish water sources and at nominal salinities during 96 h exposure of juvenile Florida pompano, *Trachinotus carolinus* L., to ammonia–nitrogen and nitrite–nitrogen

	Water source		Nominal salinity (g L^{-1})		
Parameter	Saline	Brackish	6.3	12.5	25
Calcium (mg L^{-1})	358 ± 2	56 ± 2	109 ± 1	181 ± 1	295 ± 11
Chloride (g L^{-1})	16.4 ± 0.3	0.5 ± 0.0	2.8 ± 0.1	6.0 ± 0.1	13.5 ± 0.9
Alkalinity (mg L^{-1} as $CaCO_3$)	180 ± 2	175 ± 1	175 ± 3	177 ± 5	178 ± 2

two-way repeated measures analysis of variance (ANOVA). When significant differences were detected by ANOVA, Tukey's studentized range test was used to compare treatment means. Results were considered significant at $P < 0.05$.

Results

Median-lethal concentrations of NH_3-N to juvenile Florida pompano at nominal salinities of 6.3, 12.5 and 25.0 g L^{-1} are shown in Fig. 1. No significant differences were observed in the mean LC_{50} values among salinities on a temporal basis. Mean 24, 48 and 96 h LC_{50} values ranged from 1.05 to 1.13, 1.00 to 1.08 and 0.95 to 1.01 mg NH_3-N L^{-1} respectively. Mean LC_{50} values at each salinity level tested decreased only slightly over time. No differences ($P > 0.05$) in LC_{50} at each salinity tested were observed between 24 and 48 h, and 48 and 96 h of NH_3-N exposure.

Median-lethal concentrations of NO_2-N to juvenile pompano at 24 and 48 h were significantly different at each salinity tested (Fig. 2). At 24 h, the mean LC_{50} values at salinities of 25.0, 12.5 and 6.3 g L^{-1} were 220.1, 171.1 and 67.4 mg NO_2-N L^{-1} respectively. At 48 h, the mean LC_{50} values at salinities of 25.0, 12.5 and 6.3 g L^{-1} were 140.7, 112.7 and 56.9 mg NO_2-N L^{-1} respectively. At 96 h of NO_2-N exposure, the LC_{50} values decreased as salinity declined; however, the differences were not significant. The mean values at salinities of 25.0, 12.5 and 6.3 g L^{-1} were 34.2, 26.0 and 16.7 mg NO_2-N L^{-1} respectively. With the exception of 24 and 48 h LC_{50} values at a salinity of 6.3 g L^{-1}, LC_{50} values at each salinity tested decreased significantly with the duration of exposure.

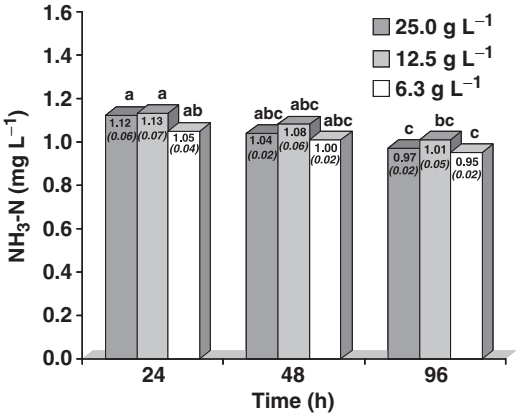

Figure 1 Median-lethal concentrations (24, 48 and 96 h LC_{50}) of ammonia-nitrogen (NH_3-N) to juvenile Florida pompano, *Trachinotus carolinus* L. (mean weight, 8.1 g) at nominal salinities of 6.3, 12.5 and 25.0 g L^{-1}. Values are means ± SE (in parentheses within bars). Bars sharing the same letter are not significantly different ($P > 0.05$).

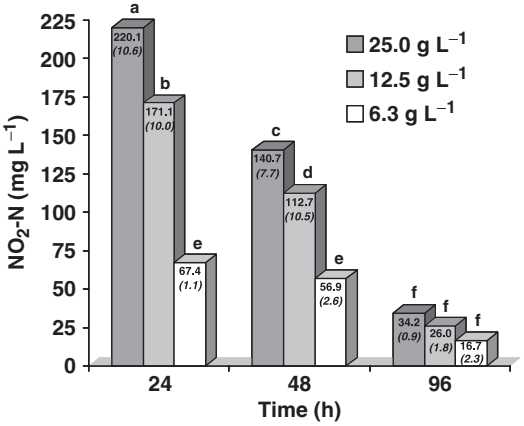

Figure 2 Median-lethal concentrations (24, 48 and 96 h LC_{50}) of nitrite-nitrogen (NO_2-N) to juvenile Florida pompano, *Trachinotus carolinus* L. (mean weight, 8.1 g), at nominal salinities of 6.3, 12.5 and 25.0 g L^{-1}. Values are means ± SE (in parentheses within bars). Bars sharing the same letter are not significantly different ($P > 0.05$).

Discussion

Median-lethal concentrations of NH_3-N to juvenile Florida pompano were not influenced by environmental salinity and were minimally affected on a temporal basis. While the latter observation was likely due to the fact that NH_3-N enters fish rapidly, the former observation was rather surprising, considering that increased salinity has been shown to reduce NH_3-N toxicity in grey mullet, *Mugil platanus* (Günther) (Sampaio *et al.* 2002), Atlantic salmon, *Salmo salar* L. (Alabaster *et al.* 1979), chinook salmon, *Oncorhynchus tshawytscha* (Walbaum) (Harader & Allen 1983) and Brazilian flounder, *Paralichthys orbignyanus* (Valenciennes) (Bianchini *et al.* 1996), presumably due to increased sodium and calcium levels. Sodium and calcium are thought to facilitate ammonia excretion via NH_4^+ and Na^+ exchange at the gill membrane (Maetz & Garcia-Romeau 1964; Soderberg & Meade 1992) and to prevent influx of NH_3-N by decreasing membrane permeability (Potts & Fleming 1970; Ogasawara & Hirano 1984). In agreement with the findings of the present study, Weirich *et al.* (1993) reported that acute tolerance of hybrid striped bass *Morone saxatilis* (Walbaum) ×

M. chrysops (Rafinesque) to environmental NH_3-N was not affected by salinities ranging from 1 to 24 g L^{-1}. However, the lack of a salinity effect in that study was likely due to deleterious physiological effects caused by unfavourable osmotic gradients of elevated salinity levels that may have obscured any benefit provided by increased calcium levels. In the present study, this effect would not be expected to occur because pompano are considered more tolerant to elevated salinities than the euryhaline hybrid striped bass. It is more likely that pompano may simply be less responsive to the beneficial effects of environmental sodium and calcium compared with other marine finfish that have been studied.

It is difficult to make a direct comparison with acute median-lethal concentrations of NH_3-N reported for other marine finfish species. However, the results of the present study indicate that pompano are relatively sensitive to environmental NH_3-N. Person-Le Ruyet, Chartois and Quemener (1995) reported the 96 h LC_{50} of NH_3-N (16–18 °C; salinity, 34–35 g L^{-1}) to juvenile European seabass, *Dicentrarchus labrax* L., gilthead seabream, *Sparus aurata* L., and turbot, *Scophthalmus maximus* L., to be 1.7, 2.5 and 2.6 mg NH_3-N L^{-1}, respectively, while Daniels, Boyd and Minton (1987) determined the 96 h LC_{50} of NH_3-N (26–27 °C; salinity, 13–14 g L^{-1}) of juvenile spotted seatrout, *Cynoscion nebulosus* (Cuvier), to be 1.7 mg NH_3-N L^{-1}.

At 24 and 48 h of exposure, median-lethal concentrations of NO_2-N to pompano were affected by salinity. Specifically, the tolerance of pompano to NO_2-N decreased with decreasing salinity over the range tested. While no significant differences due to salinity were detected for pompano exposed to NO_2-N for 96 h, the mean LC_{50} values decreased with decreasing salinity over the range tested. Our findings regarding the mitigating effect of salinity on the toxicity of NO_2-N are consistent with reports for euryhaline and marine finfish species including chinook salmon (Crawford & Allen 1977), European eel, *Anguilla anguilla* L. (Saroglia *et al.* 1981), red drum, *Sciaenops ocellatus* L. (Wise & Tomasso 1989), hybrid striped bass (Weirich *et al.* 1993), grey mullet (Sampaio *et al.* 2002) and milkfish, *Chanos chanos* (Forsskål) (Almendras 1987). In freshwater fishes sensitive to NO_2-N toxicity, NO_2-N enters the gills via an active chloride uptake mechanism (Williams & Eddy 1986) and environmental chloride competitively impedes NO_2-N uptake (Tomasso 1994; Tomasso & Grosell 2005). In contrast, marine fish utilize an intestinal chloride cotransport mechanism that has been shown to be the primary route of NO_2-N uptake (Grosell & Jensen 1999, 2000). While it is likely that environmental chloride competes for entry with NO_2-N at this site in marine fish as has been shown to occur at branchial chloride uptake sites in freshwater fish, this topic has not been investigated.

Our findings indicate that pompano are relatively sensitive to environmental NO_2-N. The 96 h LC_{50} values of NO_2-N for juvenile European seabass (17 °C; salinity, 36 g L^{-1}), and European eel (20 °C; salinity, 36 g L^{-1}) were reported by Saroglia *et al.* (1981) to be 274 and 974 mg L^{-1} respectively. Similarly, high median-lethal concentrations (48 h LC_{50}) of 248 mg NO_2-N L^{-1} (9 °C; salinity, 35 g L^{-1}) and 675 mg NO_2-N L^{-1} (28 °C; salinity 16 g L^{-1}) were reported by Crawford and Allen (1977) for chinook salmon and by Almendras (1987) for milkfish respectively. In contrast, 96 h LC_{50} values of NO_2-N for grey mullet (25 °C; salinity 30 g L^{-1}; Sampaio *et al.* 2002), southern flounder (26 °C; salinity, 0–1 g L^{-1}; Atwood, Tomasso & Smith 2001) and Brazilian flounder (25 °C; salinity, 30 g L^{-1}; Bianchini *et al.* 1996) were determined to be 36, 35 and 31 mg NO_2-N L^{-1} respectively. As demonstrated in freshwater fish (Tomasso 1994; Tomasso & Grosell 2005), species-specific differences with respect to tolerance to environmental NO_2-N of marine finfish may be due to differences in the presence or degree of chloride uptake mechanisms by which NO_2-N may be introduced (Grosell & Jensen 1999, 2000).

Conclusion

The results of this study indicate that while tolerance of pompano to NH_3-N exposure is not affected by salinity, resistance to environmental NO_2-N is compromised at reduced salinities. Culturists should be aware that this species is particularly susceptible to this toxicant, especially at lower salinities. Additional work is needed to determine the effect of chronic exposure levels on fish growth and physiological indices to establish 'safe' levels of NH_3-N and NO_2-N.

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